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THE ACTION OF CERTAIN ANTISEPTICS, TOXIC SALTS, AND ALKALOIDS ON THE BACTERIA AND PROTOZOA OF THE INTESTINE OF THE RABBIT

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In the summer of 1917, our work with some newly proposed antiseptics and recent literature regarding their properties suggested that useful conclusions might be drawn by comparing the antiseptic action of these and other typical bactericides on both bacteria and protozoa, those of the intestine of the rabbit being used for reasons stated subsequently. The work was amplified by the investigation of certain typical antiseptics, both organic and inorganic, and also of various alkaloids in current use against diseases produced by protozoa.

HISTORICAL

Ehrlich[1] has proposed as a fundamental principle of chemotherapy that the protoplasm of the parasite is equipped with specific chemoreceptors capable of combining with certain molecular configurations while the protoplasm of the host possesses fewer if any such specific receptors, and is accordingly less influenced. If the substance combining with the receptors in question be poisonous to the protoplasm, then the toxic effect will be much more evident on the parasite than on the host. This hypothesis has been supported by the observed specific action of salvarsan against treponema and spironema, and of atoxyl against trypanosoma. The idea explains the action of the salts of quinin on the plasmodium of malaria and the action of potassium antimonyl tartrate on leishmania. These effects are based on the comparative resistance of protozoa and tissue, and they were worked out some time ago. The corresponding problem of the resistance of bacteria and tissue has only recently received attention in the experiments of Carrel, Dakin, Daufresne, Dehelly and Dumas.[2]

In view of the conception of Ehrlich regarding chemoreceptors, it seems logical to regard those of human tissue and of treponema as differing considerably. The difference in the toxicity of salvarsan toward human tissue and treponema can thus be explained. The difference in the receptors of two closely related organisms, however, cannot be as great, and a substance toxic to one organism may exhibit approximately the same toxicity to others nearly allied to it.

Between bacteria and protozoa one would hardly expect extreme differences in behavior toward a toxic agent. For the demonstration of different susceptibility on the part of protozoa and bacteria to a substance injuring them, it is necessary to carefully consider such factors as the concentration and the time of action of the material tested. Pathogenic bacteria as a class are probably closely related, and while the effect of certain substances, principally dye-stuffs,

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[1] Die Experimentelle Therapie der Spirillosen, 1910.

[2] Presse méd., 1915, 23, p. 397.

has been found to vary on different varieties of bacteria, these variations are not so much in the bactericidal power of the substance employed as in the finer detail of its effect on the rate of growth, causing the inhibition of some bacterial species, while favoring the growth of others as seen in differential mediums.

In 1902, Conradi and Drigalski[3] directed the use in their medium for the isolation of the typhoid bacillus of 0.001% of crystal violet, to inhibit the growth of many other bacteria. Loeffler[4] used malachite green in a medium for the same purpose. This dye inhibits the growth of the colon bacillus while exerting no such action on the typhoid bacillus. Uhlenhuth and Xylander[5] working in another field found that antiformin—a solution of NaOCl containing some Na_2CO_3 and differing essentially in this latter constituent from Dakin's solution—while toxic to almost all bacteria exerts little effect on the tubercle bacillus, and therefore forms the basis of a method for isolating this organism in pure culture. Churchman and Michael[6] found that an addition of gentian violet in a dilution of 1 : 100,000 inhibited the growth of many bacteria, while allowing others to grow luxuriantly. These authors proposed a classification of bacteria into two groups, violet positive and violet negative on a basis of this reaction. Brown and Gilmour[7] studied the relationship between chemical constitution and bactericidal action, particularly with a view to the possible use of bactericidal dye-stuffs in clinical practice. They found that serum diminished greatly the action of such powerful bactericidal dyes as brilliant green, an effect similar to the action on mercuric chlorid under the same conditions although probably not from the same cause. Brown, Gilmour and Mackie[8] present a new method of isolating the typhoid bacillus by means of brilliant green in fluid medium. With a proper dilution of the dye only the typhoid organism will survive. R A. Lambert[9] showed that tissue cells and bacteria were killed with equal ease by potassium cyanid, tricresol, hydrogen dioxid and alcohol, but that with iodin the cells were more resistant. Inman[10] in the same year stated that the measurement of the antiphagocytic action of antiseptics was an expression of their general toxicity to living matter, and Leitch[11] uses brilliant green as a stimulant of the formation of granulation tissue. Browning, Gulbraneen, Kennaway and Thornton[12] claim that brilliant green and flavine are substances having little effect on the processes of phagocytosis, and are all the more valuable because interfering little with normal methods of combating infection; they regard this property as extremely important in determining the utility of an antiseptic to be used on infected tissue.

Generally speaking, it may be assumed that protozoa are of a more complex structure than bacteria and it might be possible to discover differences in the reaction of bacteria and of protozoa to the same antiseptic substance. Should such a condition be found, three interesting possibilities would result: (1) the possibility of separating protozoa in a new way from a culture contaminated by bacteria; (2) the possibility of removing from vaccine virus the bacteria which frequently contaminate it, and (3) the possibility of developing a method for studying the therapeutic action of the numerous preparations recommended for the treatment of human protozoan intestinal infections.

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[3] Ztschr. f. Hyg. u. Infektionskrankh., 1902, 39, p. 283.
[4] Deutsch. med. Wchnschr., 1906, 32, p. 289.
[5] Berl. klin. Wchnschr., 1908, 45, p. 1346.
[6] Jour. Exper. Med., 1912, 16, p. 822.
[7] Jour. Path. and Bacteriol., 1914, 18, p. 144.
[8] Ibid., p. 147.
[9] Jour. Exper. Med., 1916, 7, p. 683.
[10] Jour. Royal Army Med. Corps, 1916, 27, p. 500.
[11] Brit. Med. Jour., 1916, 1, p. 236.
[12] Ibid., 1917, 1, p. 73.

During the course of our work on the purification of vaccine virus the work of Krumwiede and Watson[13] appeared. These investigators have solved the problem of vaccine purification by the selective action of brilliant green in a convenient concentration against the contaminating bacteria without diminishing the viability of the virus. We think it possible that future studies with flavine may lead to similar results.

We confine ourselves in this paper to the susceptibility of bacteria and protozoa to the same toxic agents, trusting it may be possible to elaborate many of the details later.

The rabbit was selected for investigation on account of the abundant and practically constant presence of Giardia cuniculi in the intestine, generally without association with other protozoa and because of the similarity of this parasite to other species of Giardia inhabiting the intestines of rats, which have frequently been found as etiologic factors in the intestinal disorders among soldiers in trench warfare.[14]

METHODS

Chosen dilutions of the antiseptic in sterile salt solution were placed in small tubes arranged in series of 10 including a control; 0.1 c c of the contents of each tube was mixed with an equal volume of liquid material containing living protozoa and bacteria, so that the final dilution of the antiseptic was half that of the first series of tubes. Precautions were taken to insure sterile environment for the mixture. As a control, sterile salt solution was used.

Prior to mixing the dilutions of the antiseptic with the protozoa and bacteria, a series of hollow ground slides was prepared, so that as soon as the mixture had been made, a portion could be placed in a hanging drop and examined under a microscope. By making these operations at regular intervals of 1 minute, the whole series of 10 tubes could be started in 10 minutes, and by making observations 1 minute apart thereafter, the various preparations could be inspected each at 10, 20, and 30 minutes after mixing, and so on until the end of the experiment.

The microscopic preparations were studied by a strong dry lens with regard to the motility of the lamblia. When no diminution of the motility was observed, the results are recorded as +; when diminution of motility was seen, the result is recorded as ±; and when no movement could be observed either of the body or the flagella, the result is recorded as 0. At this point we assume the protozoa to be dead.

During the course of the experiments and particularly toward the close when the results are of most moment, loopfuls of the mixture of protozoa and bacteria were inoculated into sterile 1.5% acid extract broth at different times.

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[13] Jour. Infect. Dis., 1918, 22, p. 118.

[14] Fantham. H. B.. and Porter, Annie: Brit. Med. Jour., 1916, 2, p. 139.

These tubes after an incubation of 48 hours showed by growth or sterility whether or not the bacteria had been also killed at the particular time the subinoculation was made.

The mixture of protozoa and bacteria was obtained as follows: A rabbit was killed and the abdomen opened rapidly. From various parts of the small intestine, a few drops of the liquid contents were removed, and observed in a hanging drop to ascertain in which portion of the intestine the greatest number of lamblia were to be found. This part was removed from the animal and the contents collected, mixed with an equal amount of salt solution, and filtered through cotton. The filtrate contained many motile lamblia, and of course many intestinal bacteria also.

INFLUENCE OF TEMPERATURE

The observations recorded were made at room temperature, varying but slightly from 20 C. At 37.5 the action of the antiseptic is in general more rapid. Comparisons with brilliant green show that a dilution of this dye which did not kill the protozoa in 1 hour at room temperature did kill in 15-20 minutes at incubator temperature. This fact must be taken into account when it is desired to consider the action of an antiseptic in the animal body. After determining the resistance of the organisms at room temperature, a second experiment with dilutions somewhat weaker than the first must be made at 37.5 C.

OCCURRENCE OF GIARDIA CUNICULI IN THE INTESTINE OF RABBITS

In the animals used in this laboratory and which are obtained for the most part from New York, New Jersey and Pennsylvania, this protozoon is a nearly constant inhabitant of the large intestine, but is not always found in the small intestine. The occurrence seems to vary somewhat with the season, being less in winter. Large amounts of good material for this work were found in about 80% of the animals used during the summer and autumn. During the winter only about 15% contained sufficient lamblia in the small intestine to be useful.

Frequently during immunization for the preparation of agglutinating serums, especially when using bacteria of the colon-typhoid group as antigens, the animals present enteric symptoms with fluidified feces. In these cases we always have found an enormous multiplication of the protozoa in the intestine, even in winter, and this material was found specially adapted for our work. The number of protozoa in a single field of the hanging drop preparations naturally varies as outlined. Frequently such fields contained 20 or 30 organisms, and as a criterion of the applicability of the material for our work, we rejected that which when set up in a control as described did not show at least 5 actively motile lamblia during 1 minute's observation.

RESULTS

In the tables the results of this method are presented for 23 substances. The selective action on bacteria as opposed to protozoa we have found to be true of 2 proprietary antiseptics of the chlorin series (chloramin-T and "halazone"),* with one of dyes, brilliant green, and with copper in the cupric state.

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* Chloramin-T, chlorazene, and halazone are derivatives of substituted benzene-sulphonamids containing chlorin. This chlorin in many respects acts similarly to that in Dakin's solution and is the "active ingredient" of the antiseptics. That the action is modified from that of a simple hypochlorite, however, is evident from the fact that Dakin's solution (Table 1, 14) exhibits no similar selective action for bacteria.

TABLE 1

The Action of Various Antiseptic Substances in Different Dilutions on Both Protozoa and Bacteria

Antiseptic	Dilution	Time of Exposure																		Control
		10'		20'		30'		40'		50'		60'		70'		80'		90'		
		P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	
Picric acid	1:400	0	+	0	+	0	0													Alive for 160'
	1:500	0	+	0	+	0	+													
	1:700	±	+	0																
	1:800	±	+	±	+	±	+	0	+											
	1:900	+	+	+	+	±	+	±	0	0	0									
Tricresol	1:700	0		0		0														
	1:800	±	+	0	+	0	+	0	0											
	1:900	+	+	+	+	±	+	±	+	0	0									
	1:1,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	
	1:1,100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Argyrol	1:100	0		0																
	1:200	0		0																
	1:400	0		0																
	1:600	+	+	±	+	±	+	0												
	1:1,000	+	+	+	+	±	+	0												
	1:2,000	+	+	+	+	+	+	±	+	0										
Chloramin-T	1:1,400	0		0		0														Exper. a: control + for 130'. Exper. b: control ± in 85'
(Exper. b)	1:1,500	0		0		0														
	1:1,600	+	0	+	0	±	0	±	0	±	0	±	0	±	0	0				
	1:1,700	+	+	+	0	±	0	±	0	±	0	±	0	±	0	±	0			
	1:1,800	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+		
Chloramin-T	1:2,000	+		±	+	±	+	±	0	±	0	0								
(Exper. a)	1:2,600	+		+		+		±	+	±	+	±	0	0						
	1:3,000	+	+	+	+	+	+	+	+	±	+	±	+	0	+					
	1:4,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Brilliant green	1:200	0		0		0														
(Exper. a)	1:500	0		0		0														
	1:1,000	±	0	±	0	0														
	1:2,000	+	+	+	0	+	0	±	0	±	0	±	0	±	0	±	0	±	0	
	1:4,000	+	+	+	+	+	+	+	+	+	0	+	0	+	0	±	0	±	0	
Brilliant green	1:1,000	0		0	0	0	0													
(Exper. b)	1:2,000	+		+	0	+	0	+	0	+	0	+								
	1:3,000	+		+	0	+	0	+	0	+	0	+								
	1:4,000	+	+	+	+	+	+	+	+	+	0	+	0							
Copper sulphate	1:200	0	0	0	0															
(Exper. a)	1:400	0	0	0	0															
	1:1,000	0	0	0	0															
	1:2,000	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	±		
Copper sulphate	1:2,000	+	+	+	0	+	0	+												
(Exper. b)	1:3,000	+	+	+	+	+	0	+												
Halazone	1:6,000	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	±	0	Alive for 120' (+)
	1:8,000	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	±	0	
	1:10,000	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	
	1:12,000	+		+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	
	1:14,000	+		+		+	0	+	0	+	0	+	0	+	0	+	0	+	0	
	1:16,000	+		+		+	+	+	+	+	+	+	+	+	0	+		+		
	1:18,000	+		+		+		+		+		+		+	+	+	+	+	+	
Glycerol	1:4	+		±		±	+	0	+	0	+	0	+	0	0					
	1:6	+		+	+	+	+	±	+	±	+	±	+	0	+					
	1:16	+		+	+	+	+	+	+	+	+	+	+	+	+	+				
Mercuric chlorid	1:1,000	0	0	0	0	0														
	1:1,500	0	0	0	0	0														
	1:2,000	0	0	0	0	0														
	1:2,500	0	0	0	0	0														
	1:3,000	0	+	0	0	0														
Borax	1:100	+		+		±	0	±	0	±	0	0								
	1:200	+		+		+	+	+	+	±	0	±		±		0				
	1:400	+		+	+	+	+	+	+	+	+	±	+	±	+	±	+	±	+	
	1:600	+		+		+		+		+		+		+		±		±		

TABLE 1—Continued

THE ACTION OF VARIOUS ANTISEPTIC SUBSTANCES IN DIFFERENT DILUTIONS ON BOTH PROTOZOA AND BACTERIA

Antiseptic	Dilution	Time of Exposure																		Control
		10'		20'		30'		40'		50'		60'		70'		80'		90'		
		P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	
Gram's iodin solution 1:300	1:1,000	(		0	0	0														± in 80'
	1:2,000	0		0	0	0														
	1:3,000	+	+	+	0	±	0	±		0										
	1:6,000	+	+	+	+	+	0	+	0	±		±		±		0				
Barium chlorid..........	1:200	0	0	0	0	0														
	1:400	0	+	0	0	0														
	1:1,000	0	+	0	+	0	0													
	1:2,000	+	+	+	+	±	+	±	0	0										
	1:4,000	+	+	+	+	+	+	±	+	±	+	0	0							
Carbon bisulphid........ (saturated sol.)	1:2	+	+	+	+	+	+	+	+	±	0	0								
	1:5	+	+	+	+	+	+	+	+	+	0	±	0	±						
	1:20	+	+	+	+	+	+	+	+	+	+	+	+	±						
	1:50	+	+	+	+	+	+	+	+	+	+	+	+	±						
Dakin's solution.........	1:4	±		0	0	0														
	1:8	+		+	+	+	+	+	+	±	0	±	0							
	1:16	+		+		+		+		+		+	+							
	1:32	+		+		+		+		+		+	+							
Potassium antimonyl tartrate	1:500	+		±		0	+	0	+											+ in 120'
	1:1,000	+		+		±		0												
	1:1,500	+		+		+		+		±		±		±						
	1:2,000	+		+		+		+		+		+		+		+		+		
Thymol.................. (saturated sol.)	1:2	0		0	0	0														
	1:4	±		0	+	0														
	1:6	+		+		+	+	+	+	+	+	±		±						
	1:8	+		+		+		+		+		+	+	+						
Chromic acid............	1:400	0	0	0	0															
	1:800	0	0	0	0															
	1:1,200	±		0	+	0														
Phenol..................	1:200	0	0	0	0	0														
	1:220	0	0	0	+	0														
	1:240	±	+	0	+	0														
Magnesium chlorid......	1:500	+		±		±	+	0												Alive for 150' (+)
	1:1,000	+		+		±	+	±	0	0										
Ferric chlorid............	1:800	±		0	+	0														
	1:1,000	±		±		±	+	0												
	1:1,500	+		+		+		±												
Ferrous sulphate........	1:600	±		0	+	0														
	1:800	±		0	+	0														
	1:1,000	+		±	+	0														
Ethyl alcohol 95%.......	1:4	0		0		0														
	1:8	0		0		0														
	1:16	±		±		0	+													
	1:32	+		+		+		+		+	+	+	+	+	+	+	+	+	+	
Quinin sulphate..........	1:1,000	0		0	+	0														
	1:1,500	±		0		0														
	1:2,000	+		±		0														
	1:2,500	+		+		±		0												

Explanation: Column P: + = normal motility; ± = partial motility; 0 = absence of motility of the protozoa. Column B: + = the presence, or 0 = the absence of any living bacteria as shown by cultural tests.

TABLE 2

The Action of Halazone, Chloramin-T and Brilliant Green on Free Living Bacteria and a Flagellate, Colpidium Sp.

Antiseptic	Dilution	Time of Exposure														Control	
		10′		20′		30′		40′		50′		60′		70′			
		P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B
Halazone........	1:5,000	0	0	0	0											+	+
	1:10,000	0	0	0	0											+	+
	1:20,000	±		±	+	0	+									+	+
	1:40,000	+		+		+		+		+		+	+	+	+	+	+
Chloramin-T....	1:400	0		0	0											+	+
	1:600	±	+	+	+											+	+
	1:800	±	+	0	0											+	+
	1:1,000	±		0	+											+	−
	1:1,200	±		0	+											+	−
	1:1,500	+		+		+		+	+	+	+	+	+	+	+	+	−
Brilliant green...	1:3,000	0		0		0	+									+	−
	1:4,000	0		0		0	+									+	−
	1:5,000	0		0		0	+									+	−
	1:6,000	+		+		+		+		+	+	+	+	+	+	+	−

Bacteria-protozoa mixture obtained from hay infusion. The demonstration that the protozoa were living was done by both direct microscopic examination and subsequent growth in hay infusion medium.

TABLE 3

The Action of a Single Addition of Chloramin-T to Material Containing Free Living Bacteria and Protozoa (Ameba Limax sp.), and Subsequent Inoculation of the Mixtures into Semi-Solid Cabbage-Agar Medium in Sterile Petri Dishes

Antiseptic Dilution	Time of Exposure	Results After a Week Incubation at Room Temperature	
1:200	1 hour	Living bacteria but no protozoa	Only one species of sporulated bacteria
1:300	1 hour	Living bacteria but no protozoa	
1:400	1 hour	Living bacteria and protozoa	
1:500	2 hours	Living bacteria and protozoa	Several species of bacteria
1:600	2 hours	Living bacteria and protozoa	
1:700	3 hours	Living bacteria and protozoa	
1:800	3 hours	Living bacteria and protozoa	

Bacteria-protozoa mixture obtained from infusion of cabbage.

These substances are not characterized by any chemical similarity, and there is no basis for ascribing to them a common mode of action. They each probably represent a group of substances of similar action, but the variations in toxicity with slight changes in the chemical configuration we have not endeavored to work out in this paper.

Studying the action of these different substances in different concentrations against the mixture of protozoa and bacteria containing much organic matter from the intestine, we have been able to see in the case of each of these substances certain limits of dilution beyond which they are practically without action against protozoa, nevertheless the action against the bacteria is considerable. Chloramin-T in a dilution of 1 : 600 kills the bacteria in 10 minutes, while the lamblia remain alive for 70 minutes in contact with the same solution. In a dilution of 1 : 800 the bacteria are killed in 20 minutes without any harmful effect on the protozoa observable by comparing their motility with that of the control. Halazone in 1 : 6,000 kills the bacteria in 10 minutes, while in 90 minutes of contact the protozoa possess only a slightly diminished motility. A solution of 1 : 14,000 appears to be the highest dilution in which the halazone affects the bacteria. This is much more concentrated than the ordinarily accepted highest toxic dilution, as stated by the manufacturers, a result probably to be explained by the presence of the partly digested protein and other organic matter present, which combines with the available chlorin and renders it ineffective.

TABLE 4

THE ACTION OF TWO SEPARATE ADDITIONS OF CHLORAMIN-T TO MATERIAL CONTAINING FREE LIVING BACTERIA AND PROTOZOA (AMEBA LIMAX SP.), AND SUBSEQUENT INOCULATION OF THE MIXTURE INTO SEMI-SOLID CABBAGE-AGAR MEDIUM IN STERILE PETRI DISHES

Dilution After the First Addition	Exposure After the First Addition	Dilution After Second Addition (Inc. Amt. Added Previously)	Exposure After Both Additions	Results* After Second Exposure	
				P	B
(1) 1:200	90 minutes	Same. No additional Chloramin-T used	30 minutes	0	0
1:300				0	0
1:400				+	+
(2) 1:500	90 minutes	1:250	30 minutes	0	0
1:600		1:300		0	0
1:700		1:350		0	+
1:800		1:400		+	+

Bacteria-protozoa mixture obtained from infusion of cabbage.

The results of (1) and (2) by their similarity seem to indicate that failure of the chlorin antiseptic to kill bacteria is not due to its decomposition by protein, etc., bodies present, for if this were so (2) would contain more of the active antiseptic than (1) and therefore be more toxic.

* For explanation of symbols see Table 1.

Brilliant green in 1 : 1,000 kills bacteria and protozoa alike nearly instantly. In 1 : 2,000 the protozoa are apparently unaffected, while the bacteria are killed in 20 minutes. This dye manifests its bactericidal power in about the same time in a dilution of 1 : 300, still not affecting the protozoa, while in 1 : 4,000 50 minutes is required to kill all the bacteria.

Copper sulphate in a dilution between 1 : 2,000-1 : 3,000 has no harmful effect on the protozoa, although there is a visible coagulation of the organic matter present, and the bacteria are found to be dead in 20 minutes.

The intestinal bacteria used frequently contained spore-forming varieties, and, while these could be demonstrated as the last to succumb in many cases, yet their resistance was much lower than that of the Giardia. These results are contrary to the old conception that the protozoa are more sensitive to antiseptics than are bacteria. The surprising fact was also noticed that in very dilute solutions of many of the antiseptics, the motility of the protozoa was greater and persisted for a longer period than in the controls. These observations were made so consistently that we regard them as evidences of a stimulating effect of low concentrations of otherwise toxic substances.

AN ATTEMPT TO GROW GIARDIA CUNICULI IN PURE CULTURE

By selecting an antiseptic less active against protozoa and choosing dilutions and time of exposure properly, it is possible to eliminate the bacteria from a mixture with protozoa, without apparently harming the latter. We attempted to use the material so treated as a starting point for growing Giardia in pure culture. The intestinal material and antiseptic, after being mixed for a length of time sufficient to kill the bacteria, was diluted 4 or 5 times with sterile broth, and then inoculated by a capillary pipet into tubes of Noguchi medium prepared with rabbit kidney, and (in a second attempt) with fragments of rabbit intestine. These materials were sterilized by discontinuous heat, and on other occasions by contact with dilute solutions of chloramin-T and brilliant green. The tubes thus inoculated were found nearly always to remain free from bacteria. The protozoa retained their motility for 2 or 3 hours. After an incubation for 3 or 4 days, characteristic cysts of Giardia were observed, but these disappeared finally and the method accordingly did not give the hoped-for results. Similar experiments were made on semi-solid mediums containing rabbit intestine which had been partially digested with trypsin and containing traces of added glucose. Our idea was to approximate as closely as possible in vitro the conditions obtained in the small intestine of the rabbit. These experiments were also failures, however, not even a development of cysts being observed.

Giardia have heretofore never been obtained free from bacteria and it has been often suggested that some protozoa are unable to live without the presence of bacteria, and herein lies a possible explanation for our failure. We are inclined, however, to believe the fault in these attempts has not been due to Giardia, for they behaved in a perfectly normal manner for several days, but rather to our inability in devising a suitable medium for the redevelopment of the cysts into motile forms. We intend to pursue the matter farther along these lines, if possible, with other parasitic protozoa.

COMPARISON OF THE BEHAVIOR OF GIARDIA CUNICULI WITH FREE LIVING PROTOZOA

Convinced of the difficulty of growing Giardia, we attempted to apply this method to the growth of free living protozoa, which are capable of development in comparatively simple mediums. The mixture of bacteria and protozoa (Ameba limax and a flagellate) were obtained from infusions of hay and cabbage, in which the protozoa were in symbiosis with many varieties of bacteria. As shown in Tables 2, 3, and 4, however, free living protozoa are more susceptible and the accompanying bacteria less affected by the antiseptics used. Accordingly, in a dilution in which there was no difficulty in keeping the protozoa alive, it was possible to kill the bacteria. These bacteria are very sturdy saprophytic types, much more resistant than the intestinal flora. That free living protozoa show less resistance to the action of antiseptics than the parasitic types is again contrary to expectation, and is perhaps due to the comparatively simple chemical environment of the free living organism as compared to the parasite. It is certainly a state of affairs not paralleled in any sense by the bacteria, in which the saprophytic types are well recognized as the hardier.

CONCLUSIONS

In the intestinal content of the rabbit, it is possible to show that resistance of Giardia cuniculi against the action of certain antiseptic substances is markedly greater than the resistance of the intestinal bacteria. It is possible by making use of this difference in resistance to obtain Giardia free from all living bacteria, but attempts to subsequently grow them in pure culture have not succeeded.

Giardia are more resistant to the action of the toxic substances studied than are the free living protozoa studied.

A technical method has been developed which we believe will be useful in demonstrating the applicability of intestinal antiseptics against both the protozoa and the bacteria of the intestine.